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CELL ORGANIZATION AND MOVEMENT I: MICROFILAMENTS

REVIEW THE CONCEPTS

1. Actin filaments (microfilaments) are composed of monomeric actin protein subunits assembled into a twisted, two-stranded polymer. Actin filaments provide structural support, particularly to the plasma membrane, and are important for certain types of cell motility. Microtubules are composed of α- and β-tubulin heterodimers assembled into a hollow, tubelike cylinder. Microtubules provide structural support, are involved in certain types of cell motility, and help generate cell polarity. Intermediate filaments are formed from a family of related proteins such as keratin or lamin. The subunits assemble to create a strong, ropelike polymer that, depending on the specific protein, may provide support for the nuclear membrane or for cell adhesion.

2. For actin filaments, polarity refers to the fact that one end is different from the other end. This difference is generated because all subunits in an actin filament point toward the same end of the filament. The end at which the ATP-binding cleft of the terminal actin subunits contact the next internal subunits is termed the (+) end because it is the preferred end for polymerization with the lower critical concentration. The opposite end, termed the (–) end, is the less preferred end for polymerization with the higher critical concentration. Polarity can be detected by electron microscopy in "decoration" experiments in which myosin S1 fragments (essentially myosin head domains) are incubated with actin filaments. The S1 fragments bind along the actin filament with a slight tilt, leaving the actin filament decorated with arrowheads that all point toward one end of the filament. The pointed end corresponds to the (–) end and the barbed end corresponds to the (+) end.

3. Cells utilize various actin cross-linking proteins to assemble actin filaments into organized bundles or networks. Whether the actin filaments form a bundle or a network depends on the specific actin cross-linking proteins involved and the structure of the actin cross-linking protein. Actin cross-linking proteins that generate bundles typically contain a pair of tandem (i.e., closely spaced) actin-binding domains, while actin cross-linking proteins that generate networks typically contain actin-binding sites that are spaced far apart at the ends of flexible arms.

4. Once actin has been purified, its ability to assemble into filaments can be monitored by viscometry, sedimentation, fluorescence spectroscopy, or fluorescence microscopy. The viscometry method measures the viscosity of an actin solution, which is low for unassembled actin subunits but increases as actin filaments form, grow longer, and become tangled. The sedimentation assay utilizes ultracentrifugation to pellet (sediment) actin filaments but not actin subunits, and thereby separates assembled actin from unassembled actin. Fluorescence spectroscopy measures a change in the fluorescence spectrum of fluorescent-tagged actin subunits as they assemble into actin filaments. Lastly, fluorescence microscopy can be used to visualize the assembly of fluorescent-tagged actin subunits into actin filaments. Viscosity; and fluorescence microscopy will distinguish between short and long filaments, sedimentation and florescence spectroscopy will not.

5. ATP–G-actin assembles onto the ends of actin filaments and the ATP bound to the subunit is subsequently hydrolyzed to ADP and phosphate. As a result, most of the filament consists of ADP–F-actin with the exception of a small amount of ATP–F-actin at the (+) end. At the (−) end, exposed ADP–F-actin dissociates to become ADP–G-actin. If a mutation in actin prevents the protein from binding ATP, actin filaments will fail to assemble (or may assemble at very low levels if the actin can still bind ADP). If a mutation prevents actin from hydrolyzing ATP, the subunits will assemble into a filament that will be unable to disassemble normally.

6. Treadmilling is a form of actin filament assembly that occurs when the rate of subunit addition at one end equals the rate of subunit loss at the other end so that filament length remains constant as subunits flow, or treadmill, through the filament. For treadmilling actin filaments, ATP-actin subunits add to the (+) end and ADP-actin subunits dissociate from the (−) end. Treadmilling occurs when the unassembled actin subunit concentration is greater than the C_c value for the (+) end but less than the C_c value for the (−) end, which is the condition at the steady state level of polymerization.

7. Inactivation of profilin should reduce the amount of actin polymer, may disrupt signaling pathways that involve this protein, and may allow spontaneous initiation of new actin filament assembly at random places in the cell. Inactivation of thymosin-β_4 should increase the cell's free actin concentration and consequently actin filaments. Inactivation of CapZ would cause actin filaments to grow uncontrollably at the (+) end. Inactivation of Arp2/3 will inhibit assembly of actin filaments and formation of branched networks.

8. CapZ blocks the actin filament (+) end, so growth would occur only at the pointed (–) end. Tropomodulin blocks the actin filament (–) end, so growth would

occur only at the barbed end. The profilin-actin complex allows addition to a free (+) end but not to a (–) end, so growth would occur only at the barbed end.

9. Both formin and WASp are self-inhibited by intramolecular interactions before activation, and both are activated by binding of their RBD regions with an activated Rho protein. Formin is activated by binding Rho-GTP, and WASp is activated by binding Cdc42-GTP. Both proteins also contain domains that recruit profilin-actin for polymerization. The formin FH2 domain dimerizes, creating a hinged ring structure that rocks to allow addition of actin subunits while maintaining connection to the growing filament's (+) end. The WASp acidic A domain activates the Arp2/3 complex to bind to the side of an existing actin filament and initiate the growth of a new filament onto a free (+) end.

10. All myosins use energy derived from ATP hydrolysis to "walk" along actin filaments. Depending on the specific type of myosin, this movement is used to generate contraction or to transport specific cellular components relative to actin filaments. All myosins are composed of one or two heavy chains (the motor subunit) and several light chains. The heavy chains of all types of myosin have similar head domains (which interact with actin, bind and hydrolyze ATP, and generate force to move) but different tail domains, which specify the particular cellular component that a given myosin recognizes and hence the function of each myosin. For each myosin type, different light chains may be present, but all associate with the neck region just adjacent to the head domain. Myosin II is the only myosin capable of forming bipolar filaments that pull actin filaments in opposite directions.

11. Myosin motility may be observed in the sliding filament assay. In this approach, myosin motors are adsorbed onto the surface of a glass coverslip and the coverslip is placed onto a slide to create a chamber. Fluorescent-labeled actin filaments and ATP are then delivered to the chamber and the myosin motors will walk along the actin filaments. Since the motors are attached to the coverslip, the movement of myosin causes the actin filaments to slide across the coverslip surface. ATP must be added to these assays to provide the energy for myosin movement. The sliding-filament assay can reveal the direction of myosin movement if the polarity of the moving actin filaments is known. The force generated by myosin can be measured with an optical trap, in which optical forces are used to determine the force just needed to hold a sliding actin filament still.

12. The principal contractile bundles of nonmuscle cells are the circumferential belt of epithelial cells, the stress fibers present on cells cultured on artificial substrates, and the contractile ring. The circumferential belt and stress fibers appear to function in cell adhesion rather than cell movement. The contractile ring generates the cleavage furrow during cytokinesis that eventually leads to division of a single cytoplasm into two. Only myosin II can produce contractile force in these contractile bundles.

13. Conformational changes in the myosin head couple ATP hydrolysis to movement. When ATP binds to myosin, it releases from actin and hydrolyzes ATP. Upon ATP hydrolysis, the myosin head undergoes a conformational change that

rotates the head with respect to the neck portion of the protein. ADP and P_i remain bound to the myosin head. This conformational change stores the energy released by ATP hydrolysis. In the next step, the myosin-ADP-P_i complex binds a new actin subunit followed by P_i release. When P_i is released, the myosin head pops back to its original conformation, moving the bound actin filament along with its own rotation. In this way, the chemical energy of ATP hydrolysis is coupled to the mechanical work of moving an actin filament.

14. Two main structural differences between myosin II and myosin V explain the differences in their properties and functions: 1) Myosin V proteins have a longer neck than myosin II, and 2) myosin II proteins can assemble into bipolar fi laments involved in contractile functions. (Myosin V also has globular cargo binding domains at its tail.)
The neck domain acts as a lever arm for the conformational change during the power stroke. Thus, the head of myosin V moves farther than the head of myosin II with each stroke. The ATPase activity is slower for myosin V than for myosin II. Thus, the head remains bound to actin during a longer portion of each cycle. The "duty cycle" is about 70%. This means that at any time during the cycle, one or both of the heads are bound to the actin fi lament, so the vesicle does not fl oat away. As myosin V moves down a filament, its two heads follow a hand-over-hand movement. The longer duty cycle and large step size correspond with the function of myosin V, which is to provide the motor to move cargo along actin fi laments.
Bipolar complexes of myosin II work together in contraction. Hundreds of myosin heads may interact with an actin fi lament during muscle contraction. Each head remains bound only transiently, while other heads bind and move the filament. This arrangement allows for fast movement when loads are light, but it also allows the fl exibility of greater force for heavier loads. The shorter duty cycle and cooperative action between many myosin II molecules fits the function of myosin II as a contractile protein.

15. The mechanism by which a rise in Ca^{2+} triggers contractions differs in skeletal and smooth muscle. In skeletal muscle, binding of Ca^{2+} to troponin C leads to muscle contraction. Contraction of smooth muscle is triggered by activation of myosin light-chain kinase by Ca^{2+}-calmodulin.

16. By stimulating PKA phosphorylation of MLCK, albuterol inhibits the Ca^{2+}-calmodulin activation of MLCK, preventing phosphorylation of the regulatory light chain on smooth muscle myosin II that stimulates myosin activity for contraction.

17. Keratinocytes and fibroblasts have been used for experiments on cell locomotion.The movement of these cells is pictured as a series of four steps. First, the leading edge of the cell is extended by actin polymerization. Second, the newly extended membrane forms an attachment (containing embedded actin filaments) to the substrate, which anchors this portion of the cell to the substrate and prevents its retraction. Third, the bulk of the cell body is translocated for-ward, perhaps by myosin-dependent contraction of actin fi-laments. Finally, the focal adhesions at the rear of the cell are broken, perhaps by stress fiber contraction or elastic tension, so that the tail end of the cell is brought forward.

18. To study migrating cells, researchers used the wounded-cell monolayer assay. The three small GTPases (Rho, Rac, and Cdc42) orient the migrating cell as follows: Signals from the environment (such as the free space left by the scratch in the wound healing assay) are transmitted to Cdc42, which first orients the cell. The newly established cell polarity induces high Rac activity at the front of the cell, which, via activation of the Arp2/3 complex, induces the formation of lamellipodia at the leading edge. On the other hand, Rho activity is high in the rear of the cell. This induces the assembly of contractile structures such as stress fibers.

19. Traction in cell motility is provided by focal adhesions. At focal adhesions, integrins in the cell membrane bind to the substratum. Myosin-dependent cortical contraction helps to pull the cell forward. Contraction of stress fibers in the tail may help break attachments at the rear of the cell as the cell moves forward.